1 **Occurrence, antimicrobial resistance pattern and molecular characterization of**
2 ***Listeria monocytogenes* isolated from bovine's milk and meat in Mekelle City,**
3 **Ethiopia**

4 Tesfay Hailu Kidanu^{1¶}, Getachew Gugsu^{2¶*}, Yisehak Tsegaye Redda^{1¶}, Meselu Ahmed^{2¶},
5 Nesibu Awol^{2¶}

6

7 ¹College of Veterinary Sciences, Mekelle University, Mekelle, Tigray, Ethiopia

8 ²School of Veterinary Medicine, Wollo University, Dessie, Amhara, Ethiopia

9

10 * Corresponding author: Getachew Gugsu

11 E-mail; gugsag@yahoo.com

12 [¶]These authors contributed equally to this work.

13

14

15

16

17

18

19

20

21

22

23

24 Abstract

25 *Listeria monocytogenes* is an opportunistic and emerging foodborne zoonotic pathogen
 26 that encompasses a diversity of strains with varied virulence and can cause serious human
 27 and animal infections worldwide with low incidence but high hospitalization and case
 28 fatality rates. A cross-sectional study was conducted from December 2016 to June 2017
 29 to estimate the molecular epidemiology of *L. monocytogenes* and its serotypes, and
 30 antimicrobial resistance pattern of isolates in Mekelle City. A total of 768 (384 of milk
 31 and 384 meat) samples of bovine origin were collected using a purposive random
 32 sampling technique. Isolation and identification of *L. monocytogenes* was done according
 33 to standard and recommended bacteriological procedures. Genome-based confirmation of
 34 each isolate was performed at species and serovar levels by targeting *Iap*, *Imo0737*,
 35 *ORF2819* and *ORF2110* genes using specific primers. *In vitro* antimicrobial
 36 susceptibility testing was performed using agar plate antibiotic disk diffusion method.
 37 The overall prevalence of *L. monocytogenes* was 26 (3.39%). Sample type prevalence
 38 rates of *L. monocytogenes* were 4.17 % and 2.6% in meat and milk samples, respectively.
 39 There was a statically significant difference ($p < 0.05$) on the prevalence rates of the
 40 organism in meat samples collected from abattoir (1.67%), butcher shops (8.33%), and
 41 restaurants (8.33%). Serovars that were identified were belonged to 1/2b and 4b. Large
 42 proportions of isolates were highly susceptible to Ampicillin (88.46%) and Vancomycin
 43 (84.62%). However, the isolates had shown the highest level of resistance against
 44 Nalidixic Acid (96.15%). The highest intermediate was observed to Amoxicillin
 45 (57.69%). Moreover, 42.31% of the isolates were developed resistance for more than two
 46 drugs. Hence, both its occurrence and development of a multi-drug resistance indicated

47 that, a coordinated effort is imperative to reduce or eliminate the risk posed by this
48 pathogen in food chains and on controlled and careful use of antimicrobials both in
49 veterinary and human treatment regimes.

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69 Introduction

70 Food-borne diseases remain a major public health problem across the globe. The problem
71 is severe in developing countries due to difficulties in securing optimal hygienic food
72 handling practices and with the increase in consumption of raw products of animal origin.
73 Hence, food safety and particularly the safety of products of animal origin is an
74 increasingly significant issue concerning human health [1-3]. *Campylobacter species*
75 (*Campylobacter* spp.), *Salmonella* spp., *Listeria* spp., and *Escherichia coli* O157:H7 have
76 been generally found to be responsible for majority of food-borne outbreaks [4, 5]. Out of
77 the agents associated with the current worldwide increase in cases of food-borne diseases,
78 *L. monocytogenes* is a concern especially in the developing world [6].

79 *Listeria* are Gram positive, non-spore forming, non-capsular, short, regular rods, motile
80 with a characteristic tumbling movement, and with a low G+C content. The six species of
81 *Listeria* are *L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri*, and
82 *L. grayi*. However, only *L. monocytogenes* and *L. ivanovii* are considered virulent.
83 Human cases of *L. ivanovii* infection are rare [7-9], whereas *L. monocytogenes* is an
84 important food-borne pathogen [10]. *L. monocytogenes* is the causative agent of
85 listeriosis, a food-borne disease that affects mainly at risk populations such as the elderly,
86 pregnant woman, newborns and immunocompromised individuals [11].

87 *L. monocytogenes* comprises 13 serotypes with different virulence potential namely: 1/2a,
88 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7 based on somatic (O factor) and
89 flagella (H factor) antigens [12, 13]. However, more than 95% of human listeriosis
90 commonly associated with isolates that belong to serotypes 1/2a, 1/2b, and 4b, and of these

91 serotype 4b has been related to the most recent outbreaks of listeriosis, and serotypes 1/2a
92 and 4b are commonly reported in animals [14-20].

93 *L. monocytogenes* can be found in a wide variety of raw and processed foods including
94 dairy products, raw meat, vegetables, and fishery products foods of animal origin. Milk
95 and dairy products, and various meats and meat products have all been associated with
96 *Listeria* contamination and with outbreaks or sporadic cases of listeriosis [21- 24, 12].

97 *L. monocytogenes* is transmitted from man to man or from animal to man mostly through
98 ingestion of the organism with contaminated raw milk, raw meat, and dairy products.

99 Hence, these food products have been connected in several outbreaks of listeriosis [25].

100 The central characteristics of *L. monocytogenes* contributing to food-borne transmission
101 are the ability to grow as low as -0.4°C, resist heat, salt, nitrite, acidity, endure osmotic
102 stress, and survive mild preservation treatment measures commonly used to control the
103 growth of organisms in food [26]. However, in most African countries, there are a few
104 reports on *Listeria* and listeriosis, as compared to the Europe and USA [27].

105 Despite the poor surveillance programmes and lack of epidemiological data to establish a
106 comprehensive incidence rate of human listeriosis in Africa, there are a number of studies
107 that reported the occurrence of *L. monocytogenes* in foods from African countries
108 [28-31].

109 *L. monocytogenes*, as well as other *Listeria* spp., are usually susceptible to a wide range
110 of antibiotics [32]. However, *L. monocytogenes* strains resistant to one or more
111 antibiotics have been recovered from food, the environment and sporadic cases of human
112 listeriosis [33-35]. Moreover, various studies have reported *L. monocytogenes* strains that

showed multiple drug resistance to different antimicrobial substances, which is significance for the public health, therefore requiring an arduous monitoring of the antimicrobial susceptibility of *L. monocytogenes* strains [36, 37]. In Ethiopia, the actual epidemiological data about the prevalence as well as antimicrobial susceptibility pattern of *L. monocytogenes* is lacking both in the veterinary and public health sectors. Moreover, there is no published research work done on the epidemiology and antimicrobial susceptibility patterns of *L. monocytogenes* and its strain in foods of bovine origin, particularly in raw milk and meat in the current study area. Hence, this study was stipulated to estimate the molecular epidemiology of *L. monocytogenes* and its serotypes, and determine the antimicrobial susceptibility of *L. monocytogenes* from raw milk and raw meat samples of bovine origin collected from different sources in Mekelle City, Ethiopia.

Materials and Methods

Ethics approval and consent to participate

This study was reviewed and approved by the Research Ethics Committee of the College of Veterinary Sciences, Mekelle University. Owners, managers and workers of the different sites were informed on the procedures and significance of the study. Each data and analysis result was kept confidential and communicated to concerned bodies. Any participants who were not volunteers were not forced to be included.

133 Study area

134 The study was conducted from December 2016 to June 2017 in Mekelle City. Mekelle is
135 the capital city of Tigray Regional State, which is located at 39° 29' E and 13°-30' N at an
136 average altitude of 2000 m. a. s. l. and 783Km far from Addis Ababa. The climate of the
137 study area conforms to that of Ethiopian Highlands. The mean annual rainfall is 619mm,
138 and is bimodal with a short rainy season occurring from March to May and another from
139 middle September to February. The annual minimum and maximum temperature is
140 11.8°C and 29.9°C, respectively [38]. It is administratively divided into 7 sub cities and
141 is subdivided to 33 Kebeles.

142

143
144 **Fig1. Map of the study area.**

145 Study design and study population

146 A cross-sectional study was conducted from December 2016 to June 2017 in Mekelle
147 City. The study populations comprised of purposively selected milking dairy cows and
148 slaughtered cattle found in Mekelle City. The samples were collected from randomly
149 selected dairy farms, milk-shops, and cafeteria for bovine milk; and abattoir, butcher

shops, and restaurants for beef. The samples were examined to estimate the prevalence of *L. monocytogenes* and determine its antimicrobial sensitivity pattern following the standard protocols and the species and serovars were further confirmed using species-specific and serovar specific PCR primers.

Sampling size and sample collection

A total of 768 samples of bovine origin, comprised of milk (n=384) and meat (n=384), were collected using a purposive random sampling technique. The samples were collected based on the willingness of the owners until the required sample size was achieved. Thirty (30) ml of bovine milk sample was collected from dairy farms (166), shops (120), and cafeteria (98). At the same time, 25gm of bovine meat samples were collected from abattoir (240), butcher shops (84), and restaurants (60) using sterile plastic bags. All samples were labeled and transported with icebox to Molecular Biotechnology Laboratory of College of Veterinary Sciences, Mekelle University at the date of collection and stored at 4°C till further analysis was done.

Bacteriological isolation

The procedure described by the International Organization for Standardization [39] was used for milk samples. While the techniques recommended by the International Standards Organization [40, 41] and the French Association for Standardization [42] with minor modification were employed for the isolation and identification of *L. monocytogenes* from meat.

170 ***Selective enrichment***

171 The raw milk sample was thoroughly homogenized and inoculated into Listeria
172 Enrichment Broth (LEB) at 1:9 ratios [43]. After 48hrs of incubation at 30°C, the sample
173 from LEB suspensions was inoculated into Secondary Fraser Enrichment Broth (SFEB)
174 and incubated at 35°C for 24 hrs. Similarly, for the meat samples, 1:9 proportion of
175 sample to LEB was placed in sterile Stomacher plastic bags and homogenized for 1 min
176 in laboratory stomacher (Lab Belender 400, Seward Medical, London, England). Then,
177 the resulting suspension was incubated for 48 hrs. After 48hrs of incubation, 0.1ml of the
178 culture was transferred to previously prepared SFEB and then incubated for 24hrs.

179 ***Selective plating***

180 After 24hrs of incubation, aliquots of positive SFEB medium with black/dark brown or
181 dark green color was taken and streaked onto Listeria Oxford Agar plates containing
182 selective media with manufacturer's supplements and the plates were incubated at 35°C
183 for 24-48 hrs. Then, the growth of *Listeria species* on Listeria Oxford Agar plate was
184 examined.

185 ***Confirmatory tests***

186 Suspected *Listeria* colonies were sub-cultured onto Tryptose Soya Agar plate (TSA) and
187 incubated at 37°C for 18-24 hrs. Those suspected *Listeria* colonies were characterized by
188 using Gram's staining, oxidase test, characteristics of hemolysis [43], carbohydrate
189 utilization (xylose, rhamnose), and CAMP (Christie Atkins Munch Peterson) test
190 following standard methods [41- 43].

191 **Genomic DNA extraction and PCR amplification of the isolates**

192 *Genomic DNA extraction*

193 The DNA extraction was performed using the phenol-chloroform method for all the
194 isolates from samples of milk and meat according to [44].

195 *The PCR conditions*

196 Following preliminary bacteriological identification and genomic DNA extraction of
197 *L. monocytogenes* isolates, genome-based confirmation of each isolate was performed.
198 The PCR condition that was used in this study has been explained previously by [45] and
199 sequence of primers by [46] for *L. monocytogenes* and [47] for its serovars. As a positive
200 control, a standard strain of *L. monocytogenes* was used. The primers used for the PCR
201 reaction were F-3'-TTA TAC GCG ACC GAA GCC AAC-5' and R-3'-CAA ACT GCT
202 AAC ACA GCT ACT-5' for amplification of *lap gene* of *L. monocytogenes* (660 bp); F-
203 5'-AGG GCT TCA AGG ACT TAC CC-3' and R-5'-ACG ATT TCT GCT TGC CAT
204 TC-3' for amplification of *Imo0737 gene* of *L. monocytogenes* serovar 1/2a (691 bp); F-
205 5'-AGC AAA ATG CCA AAA CTC GT-3' and R-5'-CAT CAC TAA AGC CTC CCA
206 TTG-3' for amplification of *ORF2819 gene* of *L. monocytogenes* serovar1/2b (471 bp); F-
207 5'-AGT GGA CAA TTG ATT GGT GAA-3' and R-5'-CAT CCA TCC CTT ACT TTG
208 GAC-3' for amplification of *ORF2110 gene* of *L. monocytogenes* serovar 4b (597 bp).

209 Amplification was carried out with thermal cycling conditions of an initial denaturation at
210 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 45s, annealing at 50°C
211 for 1 min, and extension at 72°C for 1 min, and with a final extension at 72°C for 10 min

212 for detection of *L. monocytogenes*. But the detection of serovars was optimized with
 213 cycling conditions of an initial denaturation at 94°C for 5 minutes followed by 35 cycles
 214 of denaturation at 94°C for 30s, annealing at 54°C for 15s, and extension at 72° C for 75s,
 215 with final extension at 72°C for 10 min. Finally, the PCR products along with DNA
 216 molecular weight marker (3B Black Bio Biotech) were separated by running on a 2%
 217 (w/v) agarose gel containing 0.3mg/ml ethidium bromide. Electrophoresis was conducted
 218 in a horizontal equipment system for 120 min at 90 V using 1X TAE buffer. The
 219 amplicons were visualized under a gel documentation system and their molecular weights
 220 were estimated.

221 **Antimicrobial susceptibility testing**

222 All the 26 phenotypically and molecularly characterized isolates of *L. monocytogenes*
 223 were tested for antibiotic susceptibility pattern. The method applied for the *in vitro*
 224 antimicrobial susceptibility testing of *L. monocytogenes* isolates was the agar plate
 225 antibiotic disk diffusion method using Kirby-Bauer technique [48]. The following
 226 thirteen antibiotic disks (Himedia Laboratory Pvt limited, Mumbai, India) with their
 227 concentrations given in parentheses were used in the antibiogram testing: Amoxicillin
 228 (25µg), Ampicillin (10µg), Ciprofloxacin (5µg), Clindamycin (10µg), Cloxacillin
 229 (5µg), Erythromycin (15µg), Gentamycin (10µg), Methicillin (30µg), Nalidixic Acid
 230 (30µg), Penicillin G (10µg), Streptomycin (10µg), Tetracycline (30µg), and Vancomycin
 231 (30µg). The selection of these antibiotics was based on the availability and frequent use
 232 of these antimicrobials in the study area both in veterinary and human medicine. The
 233 results were interpreted as Susceptible (S), Intermediate (I), and Resistant (R) categories

234 based on the critical points recommended by the Clinical and Laboratory Standards
235 Institute [49].

236 **Data management and processing**

237 The data collected from the results of the laboratory investigation were entered into
238 Microsoft Excel Spreadsheet (Microsoft Corp., Redmond, WA, USA) and transferred to
239 STATA Version 11.1 for statistical analysis. Descriptive statistics, Chi-square test (χ^2)
240 and logistic regression were applied to see the associations of the food type and
241 corresponding source as risk factors with that of the occurrence of the positive result; the
242 degree of association was determined using Odds ratio (OR) and 95% confidence interval
243 (CI) as explained by [50]. For those that test, a p-value less than 0.05 was considered
244 statistically significant.

245 **Results**

246 **Overall prevalence of *L. monocytogenes***

247 The overall prevalence of *L. monocytogenes*, from the total 768 samples, was found to be
248 26(3.39%). The sample type prevalence of *L. monocytogenes* was found to be 4.17 % and
249 2.6% in meat and milk samples, respectively. In general, the prevalence of
250 *L. monocytogenes* was varied between sample types and sources. The prevalence of
251 *L. monocytogenes* in meat samples collected from abattoir, butcher shops, and restaurants
252 were 1.67%, 8.33%, and 8.33%, respectively. The result was significantly higher
253 ($p<0.05$) in butcher shops and restaurants than abattoir. Whereas, out of 384 milk samples
254 collected from dairy farms, milk shops, and cafeteria, the prevalence was 1.81%, 2.50%,
255 and 4.08%, respectively. However, there was no statically significant difference ($p>0.05$)

256 in the prevalence of *L. monocytogenes* among the various sources of milk samples (Table
257 1).

258 **Table 1. Overall prevalence of *L. monocytogenes* from different sample sources.**

Sample type	Place of collection	No. Examined	Prevalence (%)	95% CI	p-value
Milk	Farms	166	3(1.81)	0.0 - 1.00	0.531464
	Shops	120	3(2.50)	0.27 - 7.02	
	Cafeterias	98	4(4.08)	0.51 - 10.55	
	Total	384	10 (2.6)		
Meat	Abattoir	240	4(1.67)	0.27 - 4.90	0.005659
	Butchers	84	7(8.33) ^a	1.24 - 19.62	
	Restaurants	60	5(8.33)	0.84 - 17.87	
	Total	384	16 (4.17)		
Grand Total		768	26(3.39)		0.0417

259 **PCR confirmation of *L. monocytogenes* isolates and serotyping**

260 All the 26 phenotypically characterized *L. monocytogenes* isolates were also molecularly
261 confirmed as *L. monocytogenes* using *L. monocytogenes* species-specific primer, which
262 targets *Iap* gene as it is depicted below in Figure 2.

263

264 **Fig 2. PCR Product of *L. monocytogenes*; M-DNA Ladder, Lanes 1-11-**
 265 ***L. monocytogenes* (660bp) and Lane 12-Negative control.**

266 Moreover, the molecularly confirmed *L. monocytogenes* isolates were further identified at
 267 serovar levels using serovar specific primers with the help of multiplex PCR. The
 268 serovars that were identified were belonged to 1/2b and 4b as it shown below in Figure 3.

269

270 **Fig 3. PCR Products: M-DNA ladder; Lanes 1-4 and Lanes 13-15 for serovar 1/2b(471bp);**
 271 **and Lanes 5-11 for serovar 4b (597 bp); and Lane12-Negative control.**

272 Antimicrobial susceptibility pattern

273 All the 26 isolates of *L. monocytogenes* were tested for antibiotic susceptibility pattern.
 274 The antibiogram profile results indicated as large proportions of the isolates of this study
 275 were found to be highly susceptible to Ampicillin (88.46%) and Vancomycin (84.62%).
 276 However, the isolates had shown the highest level of resistance against Nalidixic Acid
 277 (96.15%). The highest intermediate was observed to Amoxicillin (57.69%) (Table 2).
 278 Moreover, 34.62%, and 42.31% of the isolates were developed resistance for two and
 279 more than two drugs, respectively, as it is shown in Figure 3.

280 **Table 2. *In vitro* antimicrobial susceptibility pattern of *L. monocytogenes* isolates.**

Antimicrobials Agents	Interpretations		
	No. of Susceptible (%)	No. of Intermediate (%)	No. of Resistance (%)
Penicillin G	13(50.00)	10(38.46)	3(11.54)
Amoxicillin	9(34.62)	15(57.69)	2(7.69)
Ampicillin	23 (88.46)	3(11.54)	0(0)
Vancomycin	22(84.62)	4(15.38)	0(0)
Ciprofloxacin	20(76.92)	6(23.08)	0(0)
Streptomycin	11(42.31)	12(46.15)	4(15.38)
Nalidixic Acid	0(0)	1(3.85)	25(96.15)
Gentamycin	21(80.77)	5(19.23)	0(0)
Methicillin	19(73.07)	7(26.92)	0(0)
Erythromycin	15(57.69)	9(34.62)	2(3.85)

Clindamycin	19(73.07)	6(14.93)	0(0)
Cloxacillin	17(65.38)	7(26.92)	2(7.69)
Tetracycline	5(19.23)	7(26.92)	14(53.85)

281

282

283 **Fig 4. *In vitro* antimicrobial multidrug resistance pattern of *L. monocytogenes***
284 **isolates.**

285 Discussion

286 The overall prevalence of *L. monocytogenes* from both sample types was found to be 26
287 (3.39%), which was characterized and confirmed both phenotypically and molecularly.
288 The current finding is in line with the findings of [51] (2.6%), [52] (2.94%), [53] (4%),
289 [30] (4.1%), [54] (4.3%), [55] (4.8%), [27] (5.1%), [56] (5.3%), [57] (5.4%), and [58]
290 (6.25%). However, it is lower than the findings of [59] (12.4%), [60] (17%), [61]
291 (26.66%), and [62] (32.7%). But it is higher than the report of [63] (1.5%).

292 The meat sample based prevalence of *L. monocytogenes* was found to be 4.17 %. This is
 293 in agreement with the findings of [57] (2.6%), [64] (3.3%), [53] (3.92%), [58] (6.66%).
 294 However, it is lower than the reports of [52] (8%), [65] (9.6%), [59] (10.3%), [66] (12%),
 295 [60] (14%), [67] (15%), [68] (15.4%), [51] (18.7%), and [69] (45.6%). But it is higher
 296 than the finding of [63] (0%). Likewise, the milk sample based prevalence of
 297 *L. monocytogenes* was found to be 2.6%. The current report is in agreement with the
 298 reports of [70] (0.41%), [62] (1.1%), [58] (4.0%), [71] (4%), [63] (4.0%), [72] (4.29%),
 299 [73] (5%), and [74] (5.1%). But it is lower than the reports of [75] (5.6%), [76] (7.54%),
 300 [57] (13%), [54] (5.3%), [52] (8%), [77] (8.8%), [51] (18.1%), [78] (21.7%), and [61]
 301 (40%). In general, the variation both in the overall and sample wise prevalence rates of
 302 *L. monocytogenes* might be due to differences in sample types of foods of bovine origin,
 303 sources of the samples, processing plants, approaches of sample collection, sample size,
 304 methodological approaches, isolation and identification techniques, prevalence
 305 calculation/interpretation, geographical locations, hygienic conditions, handling and
 306 transportation of samples, and contamination rates from utensils and personnel. The
 307 serovars that were identified in the current study were belonged to 1/2b and 4b. [73] and
 308 [69] were also reported these serovars in addition to some others. In general, the most
 309 common causes of human listeriosis among the 13 serotypes of *L. monocytogenes* are
 310 1/2a, 1/2b, and 4b, and of these serotype 4b has been related to the most recent outbreaks
 311 of listeriosis, and serotypes 1/2a and 4b are commonly reported in animals [14-20, 25].
 312 The presence of molecular serogroup 1/2b and 4b isolates (potential serotype 4b) in food
 313 of bovine origin may pose great health threat, since *L. monocytogenes* 4b has caused
 314 numerous human listeriosis outbreaks [79].

315 In the present study, the 26 *L. monocytogenes* isolates were investigated for their *in vitro*
316 antimicrobial susceptibility pattern. The antimicrobial susceptibility results indicated as
317 large proportions of the isolates were found to be highly susceptible to Ampicillin
318 (88.46%) and Vancomycin (84.62%). However, the isolates had shown the highest level
319 of resistance against Nalidixic Acid (96.15%). The highest intermediate was observed to
320 Amoxicillin (57.69%). This is in agreement with the reports of [58] who reported a high
321 degree of resistant against Nalidixic Acid (50%) and Tetracycline (37.5%) but the highest
322 level of sensitivity to Vancomycin (100%); [60] who reported 97% of susceptibility to
323 Ampicillin and Vancomycin, [65] who reported 100% of susceptibility Vancomycin, [61]
324 who reported 100% of susceptibility to Ampicillin and Vancomycin, [77] who reported
325 100% of susceptibility to Ampicillin, Gentamycin, and Vancomycin and 24.2% of
326 resistance against Tetracycline, [73] who reported a high level of sensitivity Ampicillin
327 (78.9%), Penicillin G (77%), Erythromycin (71.2%), and Vancomycin (67.3%), [62] who
328 reported 100% of susceptibility to Vancomycin and 96.4% of resistant against Nalidixic
329 Acid. This is contradicted with the findings of [80] who reported a high degree of
330 resistance against Amoxicillin (50%) and Vancomycin (50%), [76] who reported a high
331 degree of resistance against Clindamycin (100%), Ampicillin (95.7%), Erythromycin
332 (95.7%), Penicillin (91.3%), Tetracyclin (82.6%), Streptomycin (78.3%), and
333 Vancomycin (69.7%), [58] who reported a high degree of resistance against Penicillin
334 (66.7%) and the highest level of sensitivity to Amoxicillin (100%), Cloxacillin (100%),
335 and Gentamicin (100%). It is similar to the findings of [59] who reported 13.6% of
336 intermediate response to Ciprofloxacin. Moreover, 23.10%, 34.62%, and 42.31% of the
337 isolates were showed resistance for one, two, and more than two drugs, respectively. This

is lower than the findings of [76] who reported a 96.9% of multidrug resistance. But higher than the report of [58]) (16.7%), and [81] who reported a 2% of resistance to two antibiotics and 2% resistance to three antibiotics, [73] who reported that 15.3% the isolates were resistant to only one drug and 36.5% were resistant to multiple drugs. But is lower than the reports of [59] who reported 72.3% of multiple drugs resistant. In general, *Listeria monocytogenes* is usually susceptible to a wide range of antibiotics. Nevertheless, evolution of bacterial resistance towards antibiotics has been accelerated considerably by the selective pressure exerted by over prescription of drugs in clinical settings and their heavy use as promoters in animals' husbandry [35]. Moreover, the genes responsible for antibiotic resistance could be transferred through movable genetic elements such as conjugative transposons, mobilizable plasmids, and self-transferable plasmids to other foodborne bacteria in the gastrointestinal tract. In *Listeria spp.*, efflux pumps have also been reported as the resistant mechanism [82]. Hence, the use of antimicrobials in veterinary medicine is the main cause of the development of AMR foodborne bacterial pathogens including *L. monocytogenes*, as AMR pathogens can easily be transported from animal to human via food consumptions [83].

Conclusion and Recommendations

In conclusion, the current study revealed the occurrence of *L. monocytogenes* with different pathogenic serovars (1/2b and 4b) in raw meat and milk samples of bovine origin collected from different sites of the study area and development of antimicrobial resistance to one, two, and multi to the tested antimicrobials. Hence, regardless of its prevalence rate foods of bovine origin can serve as a potential vehicle for transmitting *L. monocytogenes* and can be risk of infection to consumers of raw or under cooked milk

and meat and are of significant concern for public health and give a warning signal for the possible occurrence of a foodborne outbreak in humans in the study area. It has an important public health implication that when antibiotic resistant *L. monocytogenes* is present in raw foods especially in developing countries like Ethiopia where there is very common practice of consumption of raw meat and milk products and widespread and uncontrolled use of antibiotics. As a result, both its occurrence and development of a multi-drug resistance should receive particular attention and is an alert for the concerned bodies. Thus, a coordinated effort is imperative to reduce or eliminate the risk posed by this pathogen at various stages in the food chains and on the controlled and careful use of antimicrobials both in veterinary and human treatment regimes. Moreover, awareness creation should be made on foodborne disease caused by *L. monocytogenes* with due consideration on the safe handling and consumption of foods of bovine origin and selection and safe use of antimicrobials. Finally, nationwide molecular epidemiology, phenotypic and molecular characterization, in-depth typing and drug resistant gene identification of *L. monocytogenes* should be undertaken.

Acknowledgements

The authors would like to acknowledge owners, managers and workers of the different dairy farms, abattoir, butcher shop, restaurant, milk whole seller, and cafeteria of the study site for their keen interest and cooperation during the collection of the samples, and to the College of Veterinary Sciences staff members who directly or indirectly helped us during the research period.

384 **References**

- 385 1. WHO. Foodborne Disease. A focus for Health Education; 2000.
- 386 2. Havelaar AH, Cawthorne A, Angulo F, Bellinger D, Corrigan T, Cravioto A, et al.
- 387 WHO Initiative to Estimate the Global Burden of foodborne diseases. Lancet. 2013;
- 388 381(Suppl. 2): S59.
- 389 3. Grace D. Food safety in low and middle income countries. Int J Environ Res Public
- 390 Health. 2015;12(9):10490-507. [https://doi:10.3390/ijerph120910490](https://doi.org/10.3390/ijerph120910490).
- 391 4. Alocilja EC, Radke, SM. Market analysis of biosensors for food safety.
- 392 Biosens. Bioelectron. 2003;18(5-6):841-6. [https://doi:10.1016/s0956-](https://doi.org/10.1016/s0956-5663(03)00009-5)
- 393 5663(03)00009-5.
- 394 5. Chemburu S, Wilkins E, Abdel-Hamid I. Detection of pathogenic bacteria in food
- 395 samples using highly-dispersed carbon particles. Biosens Bioelectron.
- 396 2005;21(3):491-9. [https://doi:10.1016/j.bios.2004.11.025](https://doi.org/10.1016/j.bios.2004.11.025).
- 397 6. WHO. Future trends in Veterinary public health Technical report series. 907, Report of
- 398 a WHO Study Group, Geneva; 2002. p. 1-7.
- 399 7. Seeliger HPR, Jones D. Genus *Listeria* Pierie 1940. In: Sneath, Mair, Sharpe and Holt
- 400 (eds). *Bergey's Manual of Systemic Bacteriology*. Williams & Wilkins, Baltimore 2;
- 401 1986.
- 402 8. Lovett J. Taxonomy and general characteristics of *Listeria spp*. In Miller, A.J., Smith,
- 403 J.L and Somkuti G.A. (ed.) Foodborne listeriosis. Society for Industrial
- 404 Microbiology. Elsevier Science Publishing, Inc. New York; 1990..
- 405 9. Cummins AJ, Fielding AK, McLauchlin J. *Listeria ivanovii* infection in a patient with
- 406 AIDS. Int J Infect. 1994;28(1):89-91. [https://doi:10.1016/s0163-4453\(94\)94347-8](https://doi.org/10.1016/s0163-4453(94)94347-8).

- 407 10. Swaminathan B. Foodborne pathogenic bacteria: *Listeria monocytogenes*, In: Doyle,
408 M.P., Beuchat, L.R., Montville, T.J. (Eds.), Food Microbiology: Fundamentals and
409 Frontiers, 2nd ed. ASM Press, Washington, DC; 2001.
- 410 11. Buchanan RL, Gorris LGM, Hayman MM, Jackson TC, Whiting RC. A review of
411 *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology,
412 and risk assessments. Food Control. 2017;75:1-13.
413 <https://doi.org/10.1016/j.foodcont.2016.12.016>.
- 414 12. Elliot TR, Elmer HM. *Listeria*, Listeriosis and Food Safety, 2nd Edition. CRC Press
415 Taylor & Francis Group, Boca Raton, London New York; 2007.
- 416 13. Jadhav S, Bhawe M, Palombo EA. Methods used for the detection and subtyping of
417 *Listeria monocytogenes*. J Microbiol Methods. 2012;88(3):327-41.
418 <https://doi.org/10.1016/j.mimet.2012.01.002>.
- 419 14. Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P. Differentiation of the Major
420 *Listeria monocytogenes* Serovars by Multiplex PCR. J Clin Microbiol.
421 2004a;42(8):3819-22. <https://doi.org/10.1128/jcm.42.8.3819-3822.2004>.
- 422 15. Doumith M, Cazalet C, Simoes N, Frangeul L, Jacquet C, Kunst F, et al. New
423 Aspects Regarding Evolution and Virulence of *Listeria monocytogenes* Revealed by
424 Comparative Genomics and DNA Arrays. Infect Immun. 2004b;72(2):1072-83.
425 <https://doi.org/10.1128/iai.72.2.1072-1083.2004>
- 426 16. Liu D, Lawrence ML, Wiedmann M, Gorski L, Mandrell RE, Ainsworth AJ, et al.
427 *Listeria monocytogenes* subgroups IIIA, IIIB, and IIIC delineate genetically distinct
428 populations with varied virulence potential. J Clin Microbiol. 2006;44:4229-33.
429 <https://doi.org/10.1128/JCM.01032-06>.

- 430 17. Roberts A, Nightingale K, Jeffers G, Fortes E, Kongo JM, Wiedmann M. Genetic and
431 phenotypic characterization of *Listeria monocytogenes* lineage III. Microbiology.
432 2006;152:685-93. [https://doi:10.1099/mic.0.28503-0](https://doi.org/10.1099/mic.0.28503-0).
- 433 18. Kasper S, Huhulescu S, Auer B, Heller I, Karner F, Würzner R, et al. Epidemiology
434 of listeriosis in Austria. Wien Klin Wochenschr. 2009;121(3-4):113-9.
435 [https://doi:10.1007/s00508-008-1130-2](https://doi.org/10.1007/s00508-008-1130-2).
- 436 19. Datta AR, Laksanalamai P, Solomotis M. Recent developments in molecular sub-
437 typing of *Listeria monocytogenes*. FOOD ADDIT CONTAM A. 2013;30(8):1437-
438 45. [https://doi:10.1080/19440049.2012.728722](https://doi.org/10.1080/19440049.2012.728722).
- 439 20. Mateus T, Silva J, Maia RL, Teixeira P. Listeriosis during Pregnancy: A Public
440 Health Concern. ISRN Obstet Gynecol. 2013; 2013: 1-6.
441 [https://doi:10.1155/2013/851712](https://doi.org/10.1155/2013/851712)
- 442 21. Schlech WF. Foodborne listeriosis. Clin Infect Dis. 2000;31:770-5.
443 [https://doi:10.1086/314008](https://doi.org/10.1086/314008).
- 444 22. Bell C, Kyriakides A. *Listeria*, a Practical Approach to the Organism and its Control
445 in Foods, 2nd Edn. London: Blackwell Pub; 2005.
- 446 23. Yücel N, Çitak S, Önder M. Prevalence and antibiotic resistance of *Listeria* species in
447 meat products in Ankara. Turkey. Food Microbiol. 2005;22:241-5.
448 [https://doi:10.1016/j.fm.2004.03.007](https://doi.org/10.1016/j.fm.2004.03.007).
- 449 24. Dhama K, Rajagunalan S, Chakraborty S, Verma AK, Kumar A, Tiwari R, et al.
450 Food-borne pathogens of animal origin-diagnosis, prevention, control and their
451 zoonotic significance: a review. Pak J Biol Sci. 2013;16(20):1076-85.
452 [https://doi:10.3923/pjbs.2013.1076.1085](https://doi.org/10.3923/pjbs.2013.1076.1085).

- 453 25. Kasalica A, Vukovic V, Vranjes A, Memiši N. *Listeria monocytogenes* in Milk and
454 Dairy Products. Biotechnol Anim Husbandry. 2011;27(3):1067-82.
455 <https://doi.org/10.2298/BAH1103067K>.
- 456 26. Walker SJ, Archer P, Banks JG. Growth of *Listeria monocytogenes* at refrigeration
457 temperatures. J Appl Microbiol. 1990;68(2):157-62. <https://doi.org/10.1111/j.1365-2672.1990.tb02561.x>.
- 459 27. Molla B, Yilma R, Alemayehu D. *Listeria monocytogenes* and other *Listeria species*
460 in retail meat and milk products in Addis Ababa, Ethiopia. Ethiop J Health Dev.
461 2004;18(3):208-12. <https://doi.org/10.4314/ejhd.v18i3.9962>.
- 462 28. El-Shenawy M, El-Shenawy M, Mañes J, Soriano JM. *Listeria spp.* in street-vended
463 ready-to-eat foods. Interdiscip Perspect Infect Dis. 2011;2011(968031):1-6.
464 <https://doi.org/10.1155/2011/968031>.
- 465 29. Bouayad L, Hamdi TM. Prevalence of *Listeria spp.* in ready to eat foods (RTE) from
466 Algiers (Algeria). Food Control. 2012;23(2):397-9. <https://doi.org/10.1016/j.foodcont.2011.08.006>.
- 468 30. Derra FA, Karlsmose S, Monga DP, Mache A, Svendsen CA, Félix B, et al.
469 Occurrence of *Listeria spp.* in retail meat and dairy products in the area of Addis
470 Ababa, Ethiopia. Foodborne Pathog Dis. 2013;10(6):577-9.
471 <https://doi.org/10.1089/fpd.2012.1361>.
- 472 31. Matle I, Mbatha KR, Lentsoane O, Magwedere K, Morey L, Madoroba E.
473 Occurrence, serotypes, and characteristics of *Listeria monocytogenes* in meat and
474 meat products in South Africa between 2014 and 2016. J Food Saf.
475 2019;39(4):e12629. <https://doi.org/10.1111/jfs.12629>.

- 476 32. Hof H, Nichterlein T, Kretschmar M. Management of listeriosis. Clin Microbiol Rev.
477 1997;10:345-57. <https://doi.org/10.1128/CMR.10.2.345>.
- 478 33. Hadorn K, Hachler H, Schaffner A, Kayser FH. Genetic characterization of plasmid-
479 encoded multiple antibiotic resistance in a strain of *Listeria monocytogenes* causing
480 endocarditis. Eur J Clin Microbiol Infect Dis. 1993;12:928-37.
481 <https://doi.org/10.1007/BF01992167>.
- 482 34. Franco Abuin CM, Quinto Fernandez EJ, Fente Sampayo C, Rodriguez Otero JL,
483 Dominguez Rodriguez L, Cepeda Saez A. Susceptibilities of *Listeria species* isolated
484 from food to nine antimicrobial agents. Antimicrob Agents Chemother.
485 1994;38:1655-1657. <https://doi.org/10.1128/aac.38.7.1655>.
- 486 35. Charpentier E, Gerbaud G, Jacquet C, Rocourt J, Courvalin P. Incidence of antibiotic
487 resistance in *Listeria species*. J Infect Dis. 1995;172(1):277-81.
488 <https://doi.org/10.1093/infdis/172.1.277>.
- 489 36. Lotfollahi L, Nowrouzi J, Irajian G, Masjedian F, Kazemi B, Falahat LEA, et al.
490 Prevalence and antimicrobial resistance profiles of *Listeria monocytogenes* in
491 spontaneous abortions in humans. Afr J Microbiol Res. 2011;5(14):1990-3.
492 <https://doi.org/10.5897/AJMR11.498>.
- 493 37. Altuntas EG, Kocan D, Cosansu S, Ayhan K, Juneja VK, Materon, L. Antibiotic and
494 bacteriocin sensitivity of *Listeria monocytogenes* strains isolated from different foods.
495 Food Sci Nutr. 2012;3:363-8. <https://doi.org/10.4236/fns.2012.33052>.
- 496 38. BoPED. Bureau of Planning and Economic Development: Tigray, Ethiopia; 2011.

- 497 39. ISO 11290-1. Modification of the isolation media and the haemolysis test, and
498 inclusion of precision data. ISO 11290-1:1996/Amd.1:2004. International
499 Organization for Standardization. Geneva, Switzerland; 2004.
- 500 40. ISO 11290-1. Microbiology of food and animal feeding stuffs - Horizontal method
501 for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection
502 method -Amendment 1: Modification of the isolation media and the haemolysis test,
503 and inclusion of precision data (ISO 11290-1:1996/AM1:2004); 2010.
- 504 41. ISO 11290-1. Microbiology of food and animal feeding stuffs. Horizontal method for
505 the detection and enumeration of *Listeria monocytogenes*. International Organization
506 for Standardization Part 1: Detection method. International Standard ISO 11290-1,
507 Geneva, Switzerland; 1996.
- 508 42. AFNOR. French Association for Standardization (AFNOR). Food microbiology-
509 detection of *Listeria monocytogenes* - routine method, Paris;1993.
- 510 43. Kiiyukia C. Laboratory Manual of Food Microbiology for Ethiopian Health and
511 Nutrition Research Institute food Microbiology Laboratory, UNIDO Project. Addis
512 Ababa; 2003.
- 513 44. Sambrook JR, Russel DW. Molecular Cloning: A Laboratory Manual. 3rd ed. Cold
514 Spring Harbour Laboratory Press; 2001.
- 515 45. Nayak JB, Brahmabhatt MN, Savalia CV, Bhong CD, Roy A, Kalyani IH, et al.
516 Detection and characterization of *Listeria species* from buffalo meat. Buffalo Bull.
517 2010;29(2):83-94.

- 518 46. Bubert A, Sokolovic Z, Chun SK, Papatheodorou L, Simm A, Goebel W. Differential
519 expression of *Listeria monocytogenes* virulence genes in mammalian host cells. Mol
520 Gen Genet. 1999; 261(2):323-36. <https://doi.org/10.1007/pl00008633>.
- 521 47. Bobade S, Warke S, Kalorey D. Virulence Gene Profiling and Serotyping of *Listeria*
522 *monocytogenes* from Infertility Cases of Women. Int j health sci res. 2016;6(1):440-9.
- 523 48. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a
524 standardized single disk method. Am J Clin Pathol. 1966;45(4):493-6.
525 http://dx.doi.org/10.1093/ajcp/45.4_ts.493.
- 526 49. Clinical Laboratory Standard Institute (CLSI). Performance standard for
527 antimicrobial susceptibility testing; Fourth information supplement CLSI document
528 M100-S24. CLSI, Wayne, PA; 2014.
- 529 50. Pfeiffer DU. An introduction to basic concepts of epidemiology. Royal Veterinary
530 College, Hawkshead Lane, North Mymms, Hertfordshire AL9 7TA, United
531 Kingdom; 2002.
- 532 51. Kramarenko T, Roasto M, Meremäe K., Kuningas M, Pölsama P, Elias T. *Listeria*
533 *monocytogenes* prevalence and serotype diversity in various foods. Food Control.
534 2013;30(2013):24-9. <https://doi.org/10.1016/j.foodcont.2012.06.047>.
- 535 52. Reda WW, Abdel-Moein K, Hegazi A, Mohamed Y, Abdel-Razik K. *Listeria*
536 *monocytogenes*: An emerging food-borne pathogen and its public health implications.
537 JIDC. 2016;10(2):149-54. <https://doi.org/10.3855/jidc.6616>.
- 538 53. Ndahi MD, Kwaga JKP, Bello M, Kabir J, Umoh VJ, Yakubu SE, et al. Prevalence
539 and antimicrobial susceptibility of *Listeria monocytogenes* and methicillin-resistant

- 540 *Staphylococcus aureus* strains from raw meat and meat products in Zaria, Nigeria.
- 541 Lett Appl Microbiol. 2013;58:262-9. [https://doi:10.1111/lam.12183](https://doi.org/10.1111/lam.12183).
- 542 54. Morobe IC, Obi CL, Nyila MA, Gashe BA, Matsheka MI. Prevalence, antimicrobial
- 543 resistance profile of *Listeria monocytogenes* from various foods in Gaborone,
- 544 Botswana. Afr J Biotechnol. 2009;8(22):6383-7. [https://doi:10.5897/AJB2009.000-](https://doi.org/10.5897/AJB2009.000-9486)
- 545 9486.
- 546 55. Mengesha D, Zewde BM, Toquin MT, Kleer J, Hildebrandt G, Gebreyes WA.
- 547 Occurrence and distribution of *Listeria monocytogenes* and other *Listeria species* in
- 548 ready-to eat and raw meat products. Berl Munch Tierarztl Wochenschr. 2009;122:20-
- 549 4.
- 550 56. Leong D, Alvarez-Ordóñez A, Jordan K. Monitoring occurrence and persistence of
- 551 *Listeria monocytogenes* in foods and food processing environments in the Republic of
- 552 Ireland. Front Microbiol. 2014;5:1-8. [https://doi:10.3389/fmicb.2014.00436](https://doi.org/10.3389/fmicb.2014.00436).
- 553 57. Gebretsadik S, Kassa T, Alemayehu H, Huruy K, Kebede N. Isolation and
- 554 characterization of *Listeria monocytogenes* and other *Listeria species* in foods of
- 555 animal origin in Addis Ababa, Ethiopia. J Infect Public Health. 2011;4(1):22-9.
- 556 [https://doi:10.1016/j.jiph.2010.10.00](https://doi.org/10.1016/j.jiph.2010.10.00).
- 557 58. Garedew L, Taddese A, Biru T, Nigatu S, Kebede E, Ejo M, et al. Prevalence and
- 558 antimicrobial susceptibility profile of listeria species from ready-to-eat foods of
- 559 animal origin in Gondar Town, Ethiopia. BMC Microbiol.
- 560 2015;15:100. [https://doi:10.1186/s12866-015-0434-4](https://doi.org/10.1186/s12866-015-0434-4).

- 561 59. Wang X, Lü X, Yin L, Liu H, Zhang W, Si W, et al. Occurrence and antimicrobial
562 susceptibility of *Listeria monocytogenes* isolates from retail raw foods. Food Control.
563 2013;32:153-8. <https://doi:10.1016/j.foodcont.2012.11.032>.
- 564 60. Khen BK, Lynch OA, Carroll J, McDowel, DA, Duffy G. Occurrence, Antibiotic
565 Resistance and Molecular Characterization of *Listeria monocytogenes* in the Beef
566 Chain in the Republic of Ireland. Zoonoses Public Health. 2015;62:11-7.
567 <https://doi:10.1111/zph.12106>.
- 568 61. Zafar N, Nawaz Z, Qadeer A, Anam S, Kanwar R, Ali A, et al. Prevalence, molecular
569 characterization and antibiogram study of *Listeria monocytogenes* isolated from raw
570 milk and milk products. Pure appl biol. 2020;9(3):1982-7.
571 <https://doi:10.19045/bspab.2020.90211>.
- 572 62. Rahimi E, Ameri M, Momtaz H. Prevalence and antimicrobial resistance of *Listeria*
573 species isolated from milk and dairy products in Iran. Food Control. 2010;21:1448-
574 52. <https://doi:10.1016/j.foodcont.2010.03.014>.
- 575 63. Nayak DN, Savalia CV, Kalyani IH, Kumar R, Kshirsagar DP. Isolation,
576 identification, and characterization of *Listeria spp.* from various animal origin foods.
577 Vet World. 2015;8(6):695-701. <https://doi:10.14202/vetworld.2015.695-701>.
- 578 64. Nastasijevic I, Milanov D, Velebit B, Djordjevic V, Swift C, Painset A, et al.
579 Tracking of *Listeria monocytogenes* in meat establishment using Whole Genome
580 Sequencing as a food safety management tool: A proof of concept. Int J Food
581 Microbiol. 2017;257:157-64. <https://doi:10.1016/j.ijfoodmicro.2017.06.015>.

- 582 65. Shen J, Rump L, Zhang Y, Chen Y, Wang X, Meng J. Molecular subtyping and
583 virulence gene analysis of *Listeria monocytogenes* isolates from food. Food
584 Microbiol. 2013;35(1):58-64. <https://doi.org/10.1016/j.fm.2013.02.014>.
- 585 66. Teixeira LAC, Carvalho FT, Vallim DC, Pereira RCL, Cunha Neto A, Vieira BS, et
586 al. *Listeria monocytogenes* in Export-approved Beef from Mato Grosso, Brazil:
587 Prevalence, Molecular Characterization and Resistance to Antibiotics and
588 Disinfectants. Microorganisms. 2019;8:18;
589 <https://doi.org/10.3390/microorganisms8010018>.
- 590 67. Awadallah MAI, Suelam IIA. Characterization of virulent *Listeria monocytogenes*
591 isolates recovered from ready-to-eat meat products and consumers in Cairo, Egypt.
592 Vet. World. 2014;7(10):788-93. <https://doi.org/10.14202/vetworld.2014.788-793>.
- 593 68. Indrawattana N, Nibaddhasobon T, Sookrung N, Chongsa-Nguan M, Tungtrongchit,
594 A, Makino S, et al. Prevalence of *Listeria monocytogenes* in raw meats marketed in
595 Bangkok and characterization of the isolates by phenotypic and molecular methods. J
596 Health Popul Nutr. 2011;29(1):26-38. <https://doi.org/10.3329/jhpn.v29i1.7565>.
- 597 69. Demaître N, Van Damme I, De Zutter L, Geeraerd AH, Rasschaert G, De Reu
598 K. Occurrence, distribution and diversity of *Listeria monocytogenes* contamination on
599 beef and pig carcasses after slaughter. Meat Sci. 2020;169:108177.
600 <https://doi.org/10.1016/j.meatsci.2020.108177>.
- 601 70. Rawool DB, Malik SVS, Shakuntala I, Sahare AM, Barbuddhe SB. Detection of
602 multiple virulence-associated genes in *Listeria monocytogenes* isolated from bovine
603 mastitis cases. Int J Food Microbiol. 2007;113(2):201-7.
604 <https://doi.org/10.1016/j.ijfoodmicro.2006.06.029>.

- 605 71. Muhammed W, Muleta D, Deneke Y, Gashaw A, Bitew M. Studies on Occurrence of
606 *Listeria monocytogenes* and Other Species in Milk and Milk Products in Retail
607 Market of Jimma Town, Ethiopia. J Dairy Foods. 2013;32(1):35-9.
- 608 72. Hamdi TM, Naïm M, Martin P, Jacquet C. Identification and molecular
609 characterization of *Listeria monocytogenes* isolated in raw milk in the region of
610 Algiers (Algeria). Int J Food Microbiol. 2007;116:190-3.
611 [https://doi:10.1016/j.ijfoodmicro.2006.12.038](https://doi.org/10.1016/j.ijfoodmicro.2006.12.038).
- 612 73. Kevenk TO, Gulel GT. Prevalence, Antimicrobial Resistance and Serotype
613 Distribution of *Listeria monocytogenes* Isolated from Raw Milk and Dairy Products.
614 J Food Saf. 2015;36(2016):11-8. [https://doi:10.1111/jfs.12208](https://doi.org/10.1111/jfs.12208).
- 615 74. Kalorey DR, Warke SR, Kurkure NV, Rawool DB, Barbuddhe SB. *Listeria species* in
616 bovine raw milk: A large survey of Central India. Food Control. 2008;19(2):109-12.
617 [https://doi:10.1016/j.foodcont.2007.02.006](https://doi.org/10.1016/j.foodcont.2007.02.006).
- 618 75. Seyoum ET, Woldetsadik DA, Mekonen TK, Gezahegn HA, Gebreyes WA.
619 Prevalence of *Listeria monocytogenes* in raw bovine milk and milk products from
620 central highlands of Ethiopia. JIDC. 2015;9(11):1204-9.
621 [https://doi:10.3855/jidc.6211](https://doi.org/10.3855/jidc.6211).
- 622 76. Obaidat MM, Stringer AP. Prevalence, molecular characterization, and antimicrobial
623 resistance profiles of *Listeria monocytogenes*, *Salmonella enterica*, and *Escherichia*
624 *coli* O157:H7 on dairy cattle farms in Jordan. J Dairy Sci. 2019;102(10):8710-20.
625 [https://doi:10.3168/jds.2019-16461](https://doi.org/10.3168/jds.2019-16461).
- 626 77. James O, Wuni A, Akabanda F, Jespersen L. Prevalence and Characteristics of
627 *Listeria monocytogenes* Isolates in Raw Milk, Heated Milk and Nunu, a

628 Spontaneously Fermented Milk Beverage, in Ghana. *Beverages*. 2018;4:40.
629 <https://doi.org/10.3390/beverages4020040>.

630 78. Jamali H, Radmehr B, Thong KL. Prevalence, characterisation, and antimicrobial
631 resistance of *Listeria* species and *Listeria monocytogenes* isolates from raw milk in
632 farm bulk tanks. *Food Control*, 2013;34:121-5.
633 <https://doi.org/10.1016/j.foodcont.2013.04.023>.

634 79. Ward TJ, Usgaard T, Evans P. A targeted multilocus genotyping assay for lineage,
635 serogroup, and epidemic clone typing of *Listeria monocytogenes*. *Appl Environ*
636 *Microbiol*. 2010;76:6680-4. <https://doi.org/10.1128/AEM.01008-10>.

637 80. Welekidan LN, Bahta YW, Teklehaimanot, MG, Abay GK, Wasihun AG, Dejene
638 TA, et al. Prevalence and drug resistance pattern of *Listeria monocytogenes* among
639 pregnant women in Tigray region, Northern Ethiopia: a cross-sectional study. *BMC*
640 *Res Notes*. 2019;12:538. <https://doi.org/10.1186/s13104-019-4566-8>.

641 81. Escolar C, Gómez D, Del Carmen Rota García M, Conchello P, Herrera A.
642 Antimicrobial Resistance Profiles of *Listeria monocytogenes* and *Listeria innocua*
643 Isolated from Ready-to-Eat Products of Animal Origin in Spain. *Foodborne Pathog*
644 *Dis*. 2017;14(6):357-63. <https://doi.org/10.1089/fpd.2016.2248>.

645 82. Lungu B, O'Bryan CA, Muthaiyan A, Milillo SR, Johnson MG, Crandall PG, et al.
646 *Listeria monocytogenes*: Antibiotic Resistance in Food Production. *Foodborne Pathog*
647 *Dis*. 2011;8 (5):569-78. <https://doi.org/10.1089/fpd.2010.0718>.

648 83. Conter M, Paludi D, Zanardi E, Ghidini, S, Vergara A, Ianieri, A. Characterization of
649 antimicrobial resistance of foodborne *Listeria monocytogenes*. *Int J Food Microbiol*.
650 2009;128:497-500. <https://doi.org/10.1016/j.ijfoodmicro.2008.10.018>.